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Relationship between CO_2-driven changes in extracellular acid–base balance and cellular immune response in two polar echinoderm species

S. Dupont [a,*], M. Thorndyke [b]

[a] Department of Biological and Environmental Sciences, University of Gothenburg, The Sven Lovén Centre for Marine Sciences — Kristineberg, 45178 Fiskebäckskil, Sweden
[b] The Royal Swedish Academy of Sciences, The Sven Lovén Centre for Marine Sciences — Kristineberg, 45178 Fiskebäckskil, Sweden

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ABSTRACT

Anthropogenic CO_2 emissions are acidifying the world's oceans. A growing body of evidence demonstrates that ocean acidification can impact survival, growth, development and physiology of marine invertebrates. However, little is known on the impact of elevated pCO_2 on immune-response. Here we investigate the impact of short-term (5–7 days) exposure to elevated pCO_2 (1275 μatm compared to 350 μatm in the control) on extracellular pH (pHe) and cellular immune response in two polar echinoderm species, the green sea urchin *Strongylocentrotus droebachiensis* and the seastar *Leptasterias polaris*. Both species experienced extracellular acidosis following short term exposure to elevated pCO_2. While this acidosis remained uncompensated within 7 days for *L. polaris*, pHe was fully compensated after 5 days for *S. droebachiensis*. For both species, coelomic fluid acidosis was associated with an increase in total coelomocyte number and a reduction in vibratile cells in *S. droebachiensis*. A relationship between pHe and phagocyte numbers was observed in *S. droebachiensis* suggesting a direct link between pHe and cellular immune-response. Further studies would require the coordinated effort of ecologists and immunologists to understand the role of elevated pCO_2 on the host–pathogen interactions that are involved in the stability of ecosystems.

1. Introduction

Anthropogenic CO_2 emissions are acidifying the world's oceans and a growing body of evidence demonstrates that this ocean acidification can impact survival, growth, development and physiology of marine invertebrates. Of increasing concern is the impact of ocean acidification on health status of living organisms because near-future environmental changes (e.g. global warming, ocean acidification, and increases of catastrophic meteorological events) can affect population dynamics and the distribution of pathogens. As a consequence, increases in diseases occurrence and natural population mortality could occur (Matozzo et al., 2012). Ecological immunology – the study of immune function in wild animals and how environmental stressors act to create and maintain immune system variation – is relatively new (Ellis et al., 2011; Martin et al., 2010). Surprisingly regarding the key role of immunity on species fitness, limited data are available on the impact of ocean acidification on animal immune functions. Only 4 papers consider the impact of elevated pCO_2 on invertebrate immunity. In one crustacean species, the decapod crab *Necora puber*, a 30 day exposure to elevated pCO_2 (3000 μatm) had no effect on pHe and indirect aspects of immune response (lipid peroxidation, Small et al., 2010). In the blue mussel (mollusk), a species known to experience acidosis under elevated pCO_2 (Michaelidis et al., 2005), a 32 day exposure to 1200–1400 μatm had a significant effect on the functionality of hemocytes with a decrease in phagocytosis but no effect on other aspects of the immune-response (total and differential cell counts, superoxide anion production; Bibby et al., 2008). Two bivalves species (the clam *Chamelea gallina* and the mussel *Mytilus galloprovincialis*) showed complex immune-modulation when exposed to reduced pH ($\Delta pH = 0.4$ and 0.7) and other environmental changes (temperature and salinity, Matozzo et al. 2012). To date only one paper has investigated the impact of elevated pCO_2 on an echinoderm. Hernroth et al. (2011) showed that exposure to high pCO_2 (900 μatm) for 1 week to 6 months induces a 50% reduction of coelomocyte number. Under long term exposure (6 months), immunity was further impacted (reduced phagocytic capacity, inhibited activation of p38 MAP-kinase). These changes were associated with an uncompensated acidosis in the coelomic fluid. In conclusion, previous studies have shown that species with good extracellular buffering (e.g. decapods and crabs) were not impacted while species experiencing acidosis under elevated pCO_2 (molluscs and echinoderms) also show evidence of immune-suppression. The importance of acid–base homeostasis in the maintenance of normal cellular responses and physiological integrity had long been recognized. For example, local acidosis (0.2–0.6 units lower than normal pHe) is characteristic in the interstitial fluid at infection sites in humans (Kraus and Wolf, 1996). This acidic microenvironment was suggested to play a direct influence on a broad range of immunological functions (Lardner, 2001).

* Corresponding author: The Sven Lovén Centre for Marine Sciences — Kristineberg, 45178 Fiskebäckskil, Sweden. Tel.: +46 31 786 9531.
E-mail address: sam.dupont@bioenv.gu.se (S. Dupont).

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Compensation of extracellular acid–base status (through buffering of free protons and/or pH regulation) is a key component to determine species-sensitivity to elevated pCO_2. For example, maintained performance at elevated pCO_2 has been observed in high metabolic species with a powerful ion regulatory apparatus (reviewed by Melzner et al., 2009). As a consequence, hypometabolic taxa such as calcareous sponges, corals, brachiopods, bryozoans and most echinoderms are predicted to be more sensitive. Moreover, it is often suggested that echinoderms showing poorly developed ionoregulation should be less likely to possess the physiological machinery required for effective regulation of extra-cellular acid-base status (Dielh, 1986). For example, when exposed to elevated pCO_2 (1800 μatm), the sea urchin *Psammechinus miliaris* suffered a significant reduction in coelomic fluid pH. Bicarbonate buffering was employed to reduce acidosis but no full compensation was observed within 7 days (Miles et al., 2007). A 5 day exposure to elevated pCO_2 (1350 μatm) on the sea urchin *Strongylocentrotus droebachiensis* (Drøbak, Norway, 9.5 °C) induced an uncompensated acidosis of extracellular fluids (Spicer et al., 2011). However, in a longer term experiment (Kattegat, Western Baltic,10 °C), Stumpp et al. (2012) have shown that adult *S. droebachiensis* exposed to elevated pCO_2 (1000–1400 μatm) was able to fully compensate pHe changes within 10 days by accumulation of bicarbonate and for the whole duration of the experiment (45 days). In the seastar *Asterias rubens*, pHe was significantly reduced at elevated pCO_2 (900 μatm) with no compensation observed up to 6 months of continuous exposure (Hernroth et al., 2011). As a consequence, sea urchins – given they have enough time – are more efficient at compensating extracellular acidosis than seastars.

The aim of this work was to investigate the impact of elevated pCO_2 (1275 μatm) on two echinoderm species, the seastar *Leptasterias polaris* and the sea urchin *S. droebachiensis*. Our hypotheses were that (i) sea urchins will be more efficient at compensating extracellular acidosis; (ii) cellular immune-response (estimated as total and differential coelomocyte counts) will be depressed under elevated pCO_2, and (iii) pHe and cellular immune response will be correlated.

2. Materials and methods

All experiments were performed at the Arctic Station located on the south coast of the Disko Island in central west Greenland. Adult specimens of the seastar *L. polaris* and the sea urchin *S. droebachiensis* were collected by hand in shallow (1–2 m) rocky and sandy substrates in the vicinity of Qeqertarsuaq (Disko Island, 69°15′N, 53°34′ W) in August 2009. Animals were transported to the laboratory and kept in 30 l aquariums at 10 °C using seawater from sampling site (temperature = 9 °C, salinity = 34‰, pH_{nbs} = 8.16). Animals were not fed during the experiment. Since it was not possible to aerate the seawater with air, seawater was replaced every 6 h to keep oxygen concentration higher than 90%. When bubbled with air, seawater pH_{nbs} dropped from 8.16 to 7.86 within few hours suggesting an unusually high concentration of CO_2 in the air (calculated atmospheric pCO_2 = 861 ppm based on seawater pH_{nbs} and alkalinity). A similar observation was made by Krogh (1904) and was attributed to "*a liberation of gas from the sea, such as will take place if bottom waters, possessing a high tension, should rise to the surface*".

pH and alkalinity (A_T) were measured twice a day. pH_{nbs} was measured with a Metrohm (827 pH lab) pH electrode calibrated with NIST buffers and A_T following Sarazin et al. (1999). The carbonate system speciation (pCO_2, and Ω_{Ar}) was calculated from pH and A_T using CO2SYS (Lewis and Wallace, 1998) with dissociation constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987). Our treatments were control/natural seawater ($pH_{nbs} = 8.20 \pm 0.02$, $pCO_2 = 351 \pm 20$ μatm, $\Omega_{Ar} = 2.28 \pm 0.08$) and high seawater pCO_2 ($pH_{nbs} = 7.70 \pm 0.04$, $pCO_2 = 1275 \pm 130$ μatm, $\Omega_{Ar} = 0.80 \pm 0.07$) for a temperature of 10 °C and $A_T = 2.35 \pm 37$ mM. pH was maintained in each aquarium using a computerized feedback system (Aqua-Medic) that regulates pH (NBS scale) by addition of pure gaseous CO_2 directly into the seawater (±0.02 pH units). Two replicates were used per species and pCO_2.

Everyday (7 days for *L. polaris*, 5 days for *S. droebachiensis*), more than 5 individuals of each species and for each replicate and pCO_2 were randomly collected and measured (arm length for *L. polaris* and test diameter for *S. droebachiensis*). Coelomic fluid was collected after amputation of an arm tip in *L. polaris* and after dissection of the peristome in *S. droebachiensis*. 500 μl of coleomic fluid was immediately fixed in formalin. Coelomic fluid pH (pHe, using Metrohm (827 pH lab) pH electrode) and volume (ml, using a graduated falcon tube) were then measured. Cell counts were performed by counting formalin-fixed cells in a Bürker chamber (BT, Brand, Wertheim, Germany). The measured volume was approximately 2 times 10 μl and the result is presented as cell counts per ml. All measurements were performed within 5 min of sampling. For *S. droebachiensis*, cells in coelomic fluid were characterized into 3 categories based on their morphology following Smith et al., 2010: (i) phagocytes (petaloid shape); (ii) red spherule cells (presence of red vesicles in their cytoplasm) and (iii) vibratile cells (presence of a flagellum, highly motile). For each individual, sex was determined by dissection of the gonads. For *L. polaris*, the vast majority (>95%) of coelomocyte was phagocytes/amebocytes and only this cell type was then considered.

Each mean value is expressed with its standard error of mean (mean ± SEM). Analysis of variance (ANOVA) was used to test the impact of the treatment (pCO_2). Linear regression model was used to test the relationship between variables. The Shapiro–Wilk test (1965) was used to confirm that the data were normally distributed and the Levene test was used to confirm that variances were homogenous. All data were analyzed using SAS/STAT software.

3. Results

3.1. Impact of elevated pCO_2 on the seastar *L. polaris*

The average pH_{nbs} in the coelomic fluid of the seastar *L. polaris* was 7.64 ± 0.01 in control seawater and 7.23 ± 0.01 in elevated pCO_2 seawater. A significant reduction in pHe was already observed 1 day after exposure to elevated pCO_2 with no evidence of recovery over the 7 days of the experiment (Fig. 1A).

An increase in total coelomocyte count (TCC) from 24.7 ± 0.24 to $45.4 \pm 24 \times 10^6$ cells per ml was observed after exposure to high pCO_2 and was already significant after 1 day of exposure to high pCO_2 (Fig. 1B). Fig. 1C shows the relationship between the pH decrease (ΔpHe) and the increase in TCC (relative ΔTCC) under elevated pCO_2 relative to the control. No clear relationship is identified between the two parameters but it is interesting to notice that the higher increase in TCC correspond to the higher decrease in pHe.

40% of the individuals used were females, 47% were males and it was not possible to determine the sex for the remaining 13%. No significant difference between sexes was observed for pHe (ANOVA: control, $F = 0.8$, $p < 0.68$; high pCO_2, $F = 3.87$, $p < 0.06$), and TCC (control, $F = 1.33$, $p < 0.27$; high pCO_2, $F = 1.46$, $p < 0.23$). Seastar sizes ranged between 2.5 and 8.5 cm with an average of 4.89 ± 0.16 cm for a coelomic fluid volume ranging between 1 and 20 ml and an average of 4.03 ± 0.45 ml. No significant relationship between size and pHe (linear regression: control, $F = 0.52$, $p < 0.48$; high pCO_2, $F = 0.58$, $p < 0.45$), and TCC (control, $F = 0.11$, $p < 0.74$; high pCO_2, $F = 1.51$, $p < 0.23$) was observed. A significant exponential relationship was calculated between size and coelomic fluid volume (control, $F = 34.11$, $p < 0.0001$; high pCO_2, $F = 105.74$, $p < 0.0001$) with no significant difference between treatments (ANCOVA using log (volume), $F = 0.5$, $p < 0.48$). No significant relationship between volume and pHe (linear regression: control, $F = 2.8$, $p < 0.12$; high pCO_2, $F = 0.42$,

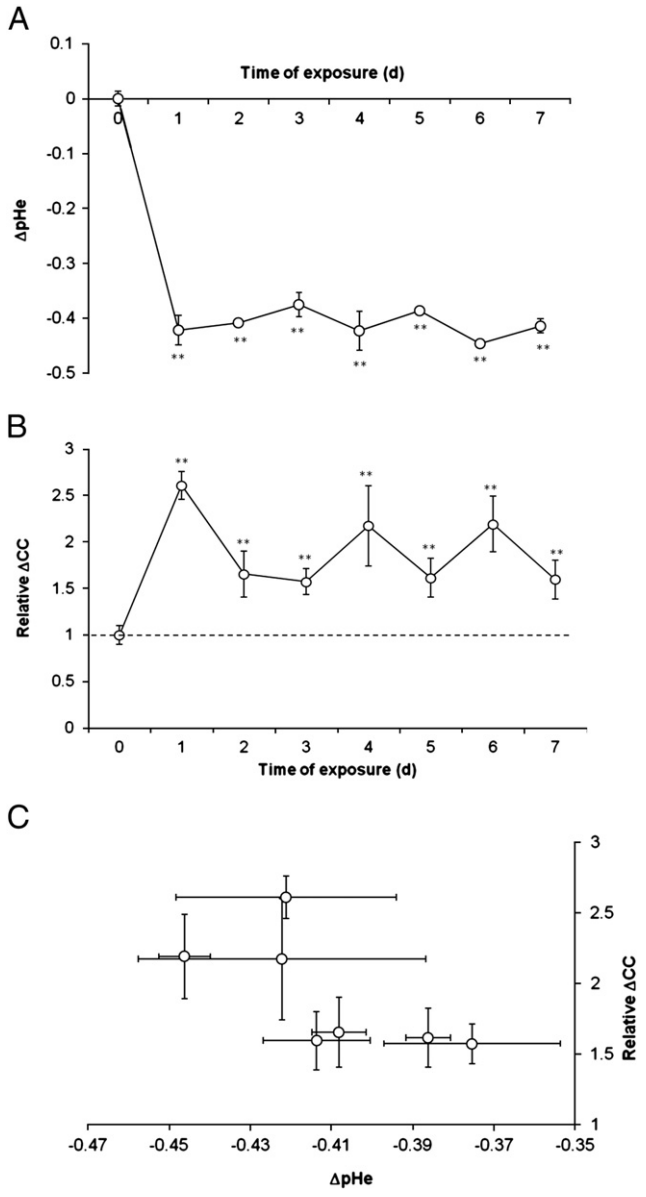

Fig. 1. Impact of elevated pCO_2 on the seastar *Leptasterias polaris*. Decrease in pHe (ΔpHe, A) and relative increase in coelomocyte cell count (relative ΔCC, B) in seastar exposed to elevated pCO_2 during the course of the experiment (7 days). C, relationship between ΔpHe and ΔCC (mean ± SEM; **p<0.01, statistically different from the control).

p<0.52), and TCC (control, F = 0.0, p<0.97; high pCO_2, F = 43, p<0.52) was observed.

3.2. Impact of elevated pCO_2 on the sea urchin *S. droebachiensis*

For the green sea urchin, the average pH_{nbs} in the coelomic fluid was 7.55 ± 0.02 in control seawater and ranged between 7.37 ± 0.03 (day 2) and 7.51 ± 0.02 (day 5) in elevated pCO_2 seawater. pHe was significantly reduced after 1 day of exposure to high pCO_2 but started to recover by day 3 and no significant difference between control and high pCO_2 was observed at day 5 (Fig. 2A).

The total coelomocyte count (TCC) ranged from 70.0 ± 12.8 to 115.6 ± 22.1 × 10^6 cells per ml. Three coelomocyte cell types were identified: (i) red spherule cells, representing 17% of the TCC; (ii) vibratile cells, representing 17% of the TTC, and (iii) phagocytes, representing 66% of the TCC. Exposure to elevated pCO_2 had an impact on the relative density of the vibratile cells (significant decrease at days 3 and 5) and phagocytes (significant increase at days 2 and 4; Fig. 2B). No significant relationships between the pH decrease (ΔpHe) and the increase in cell count (relative ΔCC) under elevated pCO_2 relative to the control were observed for red spherule (linear regression: F = 0.78, p<0.38) and vibratile cells (F = 0.01, p<0.93). However, a significant relationship (F = 5.39, p<0.02) was observed between ΔpHe and relative ΔCC for phagocytes (Fig. 2C); a larger number of phagocytes being present in the coelomic fluid when the acidosis is the more severe.

The ratio of males and females used in this study was 4:1. No significant difference between sexes was observed for pHe (ANOVA: control, F = 3.44, p<0.10; high pCO_2, F = 3.07, p<0.07), red spherule CC (control, F = 1.40, p<0.27; high pCO_2, F = 2.98, p<0.09), vibratile CC (control, F = 1.29, p<0.28; high pCO_2, F = 1.00, p<0.32) and phagocytes (control, F = 0.05, p<0.83; high pCO_2, F = 0.75, p<0.38).

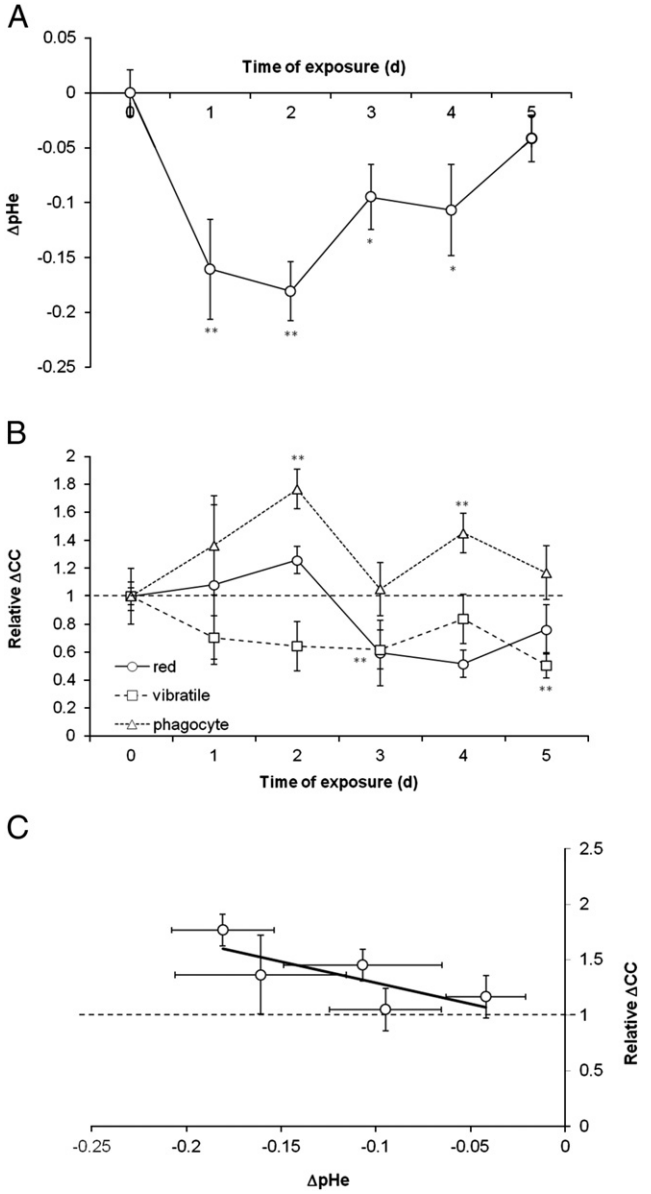

Fig. 2. Impact of elevated pCO_2 on the sea urchin *Strongylocentrotus droebachiensis*. Decrease in pHe (ΔpHe, A) and relative increase in coelomocyte cell count (relative ΔCC, B) in seas urchin exposed to elevated pCO_2 during the course of the experiment (5 days). C, relationship between ΔpHe and ΔCC for phagocytes (mean ± SEM; *p<0.05, **p<0.01, statistically different from the control).

Sea urchin sizes ranged between 3.5 and 7.5 cm with an average of 5.66 ± 0.13 cm for a coelomic fluid volume ranging between 2.5 and 38 ml and an average of 11.14 ± 0.98 ml. No significant relationship between size and pHe (linear regression: control, $F=0.10$, $p<0.76$; high pCO_2, $F=0.80$, $p<0.68$), red spherule CC (control, $F=0.10$, $p<0.76$; high pCO_2, $F=0.77$, $p<0.39$), vibratile CC (control, $F=2.85$, $p<0.13$; high pCO_2, $F=0.05$, $p<0.82$) and phagocytes (control, $F=2.62$, $p<0.14$; high pCO_2, $F=0.03$, $p<0.87$) was observed. A significant exponential relationship was calculated between size and coelomic fluid volume (control, $F=40.27$, $p<0.0001$; high pCO_2, $F=59.92$, $p<0.0001$) with no significant difference between treatments (ANCOVA using log (volume), $F=0.22$, $p<0.64$). No significant relationship between volume and pHe (linear regression: control, $F=0.04$, $p<0.82$; high pCO_2, $F=0.00$, $p<0.98$), red spherule CC (control, $F=5.34$, $p<0.05$; high pCO_2, $F=0.74$, $p<0.40$), vibratile CC (control, $F=1.81$, $p<0.21$; high pCO_2, $F=0.00$, $p<0.99$) and phagocytes (control, $F=5.03$, $p<0.05$; high pCO_2, $F=2.64$, $p<0.11$) was observed.

4. Discussion

Our results showed that elevated pCO_2 induced extracellular acidosis with consequences on the cellular immune response. Differences between the two studied species were also observed, the green sea urchin *S. droebachiensis* was able to fully compensate for acidosis within 5 days but no compensation was observed for the seastar *L. polaris* after 7 days.

4.1. Acid–base balance

Our results showing no compensation in the seastar *L. polaris* are consistent with the similar observation made by Hernroth et al. (2011) on another seastar *A. rubens*. A constant $\Delta pHe = 0.15$ from 7.5 to 7.35 was observed when *A. rubens* was exposed to 900 μatm for up to 6 months. In *L. polaris*, the pHe decrease was more pronounced, dropping from 7.64 to 7.23 when exposed to 1275 μatm. In both seastar species, the rate of proton-equivalent ion exchange between the coelomic fluid and ambient seawater was maintained at the same level over time in both normal and acidified conditions.

In the sea urchin *S. droebachiensis*, pHe decreased from 7.55 to 7.37 within one day of exposure to high pCO_2 but started to recover after 3 days reaching full compensation at day 5. This is contrasting with previous research on sea urchins (Miles et al., 2007; Spicer et al., 2011). In the same species, Spicer et al. (2011) showed a similar decrease in the pHe from 7.55 to 7.3 when exposed to 1350 μatm. However, Stumpp et al. (2012) showed a decrease in pHe from 7.7 to 7.4 within 4 days of exposure to 1400 μatm with evidence of compensation after day 4 and full compensation at day 10. These differences could be a consequence of intra-specific differences (see Dupont et al., 2010 for discussion). However, it is also possible that the absence of compensation observed in Miles et al. (2007) and Spicer et al. (2011) is a consequence of too short term experiment, not allowing enough time to compensate in the tested environmental conditions.

All together, these results suggest that sea urchins are more efficient than seastars at compensating for acidosis when exposed to elevated pCO_2. Stumpp et al. (2012) showed evidence of bicarbonate accumulation in the compensation process in sea urchin and the skeleton may then play a major role in species ability to regulate pHe. The mature skeleton of adult echinoderms varies greatly according to class. Echinoderms with substantial skeletal structure with high level of organization such as echinoids, ophiuroids and crinoids may then be more efficient at compensating pHe in elevated pCO_2 conditions than species having more flexible and delicate skeletons comprising small calcite ossicles embedded in a connective tissue matrix such as Holothuroids and Asteroids.

4.2. Immune system

Elevated pCO_2 also had an impact on coelomocytes, increasing the total number of coelomocytes in the seastar *L. polaris* and increasing the number and phagocytes and decreasing the number of vibratile cells in the sea urchin *S. droebachiensis*. This is somewhat contrasting with the only report on the impact of high pCO_2 on another echinoderm species. Chronic exposure to 900 μatm induced a 2 fold reduction in total coelomocyte count in the seastar *A. rubens* (Hernroth et al., 2011).

Echinoderms are long lived animals displaying a remarkable ability to heal wounds and combat major infections (Smith et al., 2010). Their defense systems are extremely sensitive and respond rapidly to even minor perturbations (Smith and Davidson, 2011). The body cavity of echinoderms is filled with coelomic fluid, which bathes the internal organs. The echinoderm immune system is comprised of two parts: a cellular and a humoral response. Humoral activity is mediated by a variety of compounds which are secreted within the coelomic fluid and play a role in defense against infection (Ramírez-Gomez and Garcia-Arrarás, 2010). The coelomic fluid also forms a complex tissue containing abundant and morphologically heterogeneous coelomocytes. More than 10 cell types have been described (see Ramírez-Gomez and Garcia-Arrarás, 2010). One of the most present cell-type is the phagocyte. Their main characteristic is their ability to phagocyte other cells and foreign particles. Spherule cells are found mostly in urchins and are characterized by the presence of vesicles in their cytoplasm. They have been associated with antibacterial activity, inflammatory responses, extracellular matrix remodeling and wound healing. Another cell type present in sea urchins is the vibratile cells. These highly motile cells possess a unique flagellum and may play a role in coelomic fluid movement and clotting. The proportion of each cell type is taxa specific. In seastars, the vast majority (>90%) of coelomocyte is phagocytes/amebocytes. In sea urchin, coelomocytes comprises phagocytes (40–80%), spherule cells (7–40%) and vibratile cells (11–20%, Ramírez-Gomez and Garcia-Arrarás, 2010). The proportion of cells observed in our study fitted these values.

Coelomocyte type's distribution is very dynamic, changing according to the physiological or immune state, making them useful sensors of environmental stresses (Smith et al., 2010). For example, in the seastar *A. rubens* amebocytes increase in number after injection of bacteria (Coteur et al., 2002). In the sea urchin *S. droebachiensis*, the concentration of coelomocytes decreased after infection due to smaller numbers of spherule and vibratile cells, with no difference in phagocyte cells (Jellet et al., 1988). In our experiment, we observe an increase in amebocytes in seastar and in phagocytes in sea urchin when exposed to elevated pCO_2. These coelomocytes play a key role in phagocytosis suggesting a potential up-regulation of the cellular immune response. However, consequences for the immune response are difficult to assess without considering exposure to pathogens. For example, it was shown in two species including a seastar that high pCO_2 had a negative effect on phagocytic activity (Bibby et al., 2008; Hernroth et al., 2011). As a consequence, an increase in cell number does not necessarily imply an increase in cellular immune response efficiency. On the other hand, the observed decreased in vibratile cells in the sea urchin *S. droebachiensis* could also have non-immunological negative effects. Another related caveat related to the false assumption that immunologically, "more is better" and that immunosuppression is related to a decrease in cell number or performance. Immuno-competence should rather be measured and define functionally, from the perspective of fitness and resource allocation (Viney et al., 2005). For example, experimental design should include the study of survival after pathogen exposure.

Coelomocyte cells also play a key role in clotting wound healing and regeneration. For example, arm regeneration in brittlestars is an epimorphic process in which new structures develop from a blastema formed from an accumulation of cells,

including phagocytes and undifferentiated coelomocytes (Biressi et al., 2010). As a consequence, an increase in coelomocyte number under elevated pCO_2 may play a role in the increased regeneration rate observed under high pCO_2 in the brittlestar *Amphiura filiformis* (Wood et al., 2008).

4.3. Consequences for tested species and echinoderms

Only sublethal impacts on pHe and coelomocyte numbers were observed and it is then very difficult to make any prediction on the potential impact of elevated pCO_2 on the studied species or on echinoderms. Regarding the importance of this group, more research is needed to understand the long term consequences of the observed acidosis and changes in immune cell response. Echinoderms are among the most abundant and ecologically successful group of the marine organisms and of tremendous ecological and economical importance (Micael et al., 2009). This is also true for the two species used in this study. The 6-armed arctic seastar *L. polaris* is a major predator of subtidal communities. Juveniles and small adults (>15 cm in diameter) are mostly found on rocky substrates in shallow water where they feed on small bivalves *Mytilus edulis* and *Hyatekka arctica*. Adults (>15 cm) occupy cobble to sand and mud areas and feed on large infaunal bivalves and epibenthic gastropods (Rochette et al., 1994). The green sea urchin *S. droebachiensis* is a widely distributed calcifier playing a key ecological and economical role in boreal coastal ecosystems. For example, on European coasts, grazing by the green sea urchin is central for structuring marine benthic communities (Norderhaug and Christie, 2009). In some areas, it has been considered as a pest as intensive grazing destroyed kelp habitats and limited the production of commercial species such as cod (Kålås et al., 2006). On the other hand, it is fished and now cultured for roes in the Northwest Atlantic and Northeast Pacific (Vadas et al., 2000).

Echinoderms are one of the primary targets in ocean acidification studies (Dupont et al., 2010). However, only sub-lethal effects of high pCO_2 on adults have been demonstrated (Dashfield et al., 2008; Findlay et al., 2011; Hernroth et al., 2011; Siikavuopio et al., 2007; Stumpp et al., 2012; Wood et al., 2008, 2010), even over a 16 month exposure to 1200 µatm in the green sea urchin *S. droebachiensis* (Dupont et al., 2012). Echinoderm immune biology originates from the aquaculture field where many diseases and infections were observed. However, immunity can play an important role in stable maintenance of echinoderm populations. Mass mortality and population crash due to contamination by pathogen have been documented. For example, outbreaks and mass mortality of *S. droebachiensis* have been observed along the Atlantic coast of Nova Scotia (Scheibling and Henningar, 1997) with a resulting major impact on the ecology of this area (Scheibling, 1986).

4.4. Consequences for polar species

Early in ocean acidification research, concerns have been expressed on the effect of elevated pCO_2 on species from high-latitudes (Orr et al., 2005). Species living in cold water Polar Regions exhibit a lower metabolism and are already exposed to high levels of pCO_2 as a result of temperature and upwelling of CO_2-rich seawaters (Feely et al., 2004). Models predict that the surface water of the polar oceans will be the first to be under-saturated with respect to aragonite as early as within a decade for the Arctic Ocean (Steinacher et al., 2009). Echinoderms with their high-magnesium calcite skeleton are sometime seen as the "canary in the coal mine" in Polar Regions (McClintock et al., 2009). However, available information, including our results only showing sub-lethal effects of elevated pCO_2, does not demonstrate an increased sensitivity in Polar species (see also Wood et al., 2011).

5. Conclusions

Our results fit several of our hypotheses: sea urchins were more efficient at compensating extracellular acidosis than seastars; and our results showed an intriguing relationship between pHe and phagocyte numbers in the sea urchin *S. droebachiensis*. However, cellular immune-response (estimated as total and differential coelomocyte counts) under elevated pCO_2 was more complex than predicted with both increase and decrease of coelomocyte types. No lethal effect was observed but changes in immunity, resistance to pathogen and clotting/regeneration is likely. Further studies would require the coordinated effort of ecologists and immunologists to understand the role of elevated pCO_2 on host–pathogen interactions that is involved in the stability of ecosystems. This will be of high importance in near-future marine ecosystems facing increasing incidence of disease in various marine organisms, including echinoderms, due to global warming and catastrophic meteorological events (e.g. storms, hurricanes; Scheibling and Lauzon-Guay, 2010).

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